K083391

Thyretain™ TSI Reporter BioAssay
SECTION 05, 510(K) SUMMARY

DIAGNOSTIC HYBRIDS
Integrating Science and Humanity

APPLICANT

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MAY 21 2009

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DATE OF PREPARATION OF 510(k) SUMMARY

November 14, 2008

DEVICE NAME

Trade name: Thyretain ™ TSI Reporter BioAssay
Common name: TSI Reporter
Classification name: System, Test, Thyroid Autoantibody
Product Code: JZO
Regulation: 21 CFR § 866.5870, Thyroid Autoantibody Immunological Test

DEVICE DESCRIPTION

The Thyretain ™ TSI Reporter BioAssay (TSI Reporter) utilizes a patented bioassay technology to detect thyroid stimulating immunoglobulin (TSI) in human serum. Genetically engineered Chinese hamster ovary (CHO) cells, expressing a chimeric form[1] of the human thyroid stimulating hormone receptor (TSHR) and a cyclic adenosine monophosphate (cAMP) induced luciferase reporter gene, are cryogenically preserved and provided in measured aliquots. The CHO Mc4 cell line has a nucleic acid sequence encoding a chimeric human TSH receptor, designed for reduced response to thyroid blocking immunoglobulin (TBI) activity. Thus, the hTSH receptor, comprised of 730 amino acids, has amino acid residues 262 to 335 replaced by the equivalently located 73 amino acid residues of the rat Luteinizing Hormone receptor to form the chimeric TSHR. This chimeric receptor is linked to a firefly luciferase reporter gene in operable combination with a glycoprotein hormone α-subunit promoter.

The cells are seeded and grown for 15 to 18 hours to confluent monolayers in a 96-well plate. Patient sera, reference control, positive and normal controls and are diluted with a proprietary reaction buffer (RB), added to the cell monolayers and allowed to react with the cells for 3 hours. During this induction period TSI, if present in the patient serum, bind to the chimeric human TSHR on the cell surface. This binding event induces a signaling cascade resulting in increased production of intra-cellular cAMP. This increased production of cAMP is evidenced by increased production of luciferase. At the conclusion of the 3 hour induction period the cells are lysed. Luciferase levels are then measured using a luminometer. A significant increase in luminescence over the Reference Control indicates the presence of TSI antibodies in the sample.

KIT COMPONENTS

1. **CHO Mc4 *FreshFrozenCells*®:** Cryovials containing CHO Mc4 cells cryogenically preserved in cryoprotective medium containing DMSO. Reagent is stored at -70°C or lower.
2. **Cell Attachment Solution**, 100-mL: A proprietary reagent used to treat the wells of a 96-well plate prior to planting the cells that promotes rapid cell attachment. Reagent is stored at 15° to 30°C.
3. **Growth Medium,** 100-mL: Hamm's F-12 cell culture medium containing 10% FBS. Reagent is stored at 2° to 8°C.
4. **Reaction Buffer**, 500-mL: A proprietary buffer that augments the reaction of TSI with the TSHR. Reagent is stored at 2° to 8°C.
5. **Control Set:**
    a. **Positive Control,** 0.5-mL: TSI-containing human serum which yields a value that is ≥140% of the Reference Control. Reagent is stored at -70°C or lower.
    b. **Reference Control,** 0.5-mL: A bTSH-containing solution against which Controls and Test Specimens are compared. Reagent is stored at -70°C or lower.
    c. **Normal Control**, 0.5-mL: Human serum that is negative for the presence of TSI which yields a value that is <140% of the Reference Control. Reagent is stored at -70°C or lower.
6. **Luciferase Assay Reagent Set:**
    a. **Luciferase Substrate**, 1 vial: A lyophylized beetle luciferin substrate which is converted by luciferase to oxyluciferin and light. Reagent is stored at -20°C or lower.
    b. **Luciferase Assay Buffer Solution**, 1 vial, 10-mL: A cell culture lysis buffer.

INTENDED USE

The Thyretain ™ TSI Reporter BioAssay is intended for the qualitative detection in serum of thyroid stimulating autoantibodies to the thyroid stimulating hormone (TSH) receptors (TSHRs) on the thyroid. The detection of these stimulating autoantibodies, in conjunction with other clinical and laboratory findings, may be useful as an aid in the differential diagnosis of patients with Graves' disease (GD).

Legally marketed device to which equivalence is claimed:

k032134 KRONUS TSH Receptor Antibody (TRAb) Coated Tube (CT) Assay Kit

Intended Use: The KRONUS TSH Receptor Antibody (TRAb) Coated Tube (CT) Assay Kit is designed to measure human serum autoantibodies to the thyroid stimulating hormone (TSH or thyrotropin) receptor. The TRAb CT kit is useful as an aid in the differential diagnosis of Graves' Disease.

Table 5.1: Subject Device and Predicate Device Characteristics

Similarities		
Item	**Subject Device**	**Predicate Device**
Intended Use	The Thyretain ™ TSI Reporter BioAssay is intended for the qualitative detection of thyroid stimulating autoantibodies to the thyroid stimulating hormones receptors (TSHRs) on the thyroid. The detection of these stimulating autoantibodies, in conjunction with other clinical and laboratory findings, may be useful as an aid in the differential diagnosis of patients with Graves' disease.	KRONUS TSDH Receptor Antibody (TRAb) Coated Tube (CT) Assay Kit is designed to measure human serum autoantibodies to the thyroid stimulating hormone (TSH or thyrotropin) receptor. The TRAb CT kit is useful as an aid in the differential diagnosis of Graves' disease.
Sample matrix	Serum	same
Differences		
Item	**Subject Device**	**Predicate Device**
Trade name	Thyretain ™ TSI Reporter BioAssay	KRONUS TSH Receptor Antibody (TRAb) Coated Tube (CT) Assay Kit
Assay Format	Qualitative	Qualitative and quantitative
Assay principle	Cell-based Chemiluminescent	Radioreceptor Assay

Table 5.1: Subject Device and Predicate Device Characteristics		
Differences		
Item	**Subject Device**	**Predicate Device**
Solid Phase	CHO Mc4 cell monolayer (96-well microplate)	TSHR-coated tube
Analyte	Thyroid Stimulating Immunoglobulin to Thyroid stimulating hormone (TSI-hyperthyroidism)	TSHR antibodies including both Thyroid stimulating hormone (TSI-hyperthyroidism) and blocking hormone (TBI-hypothyroidism)
Calibration	NIBSC Standard 03/192 or similar standard	NIBSC Standard 90/672 or similar calibrator
Signal	Relative Light Units	Radioactivity
Detection instrument	Luminometer	Gamma counter set for ^{125}I
Cut-off	Positive: ≥ 140% of Reference Control Negative: < 140% of Reference Control	Positive: 15% inhibition Indeterminate: 11-15% inhibition Negative: <11% inhibition

NON-CLINICAL PERFORMANCE

1. **Limit of Detection**
   The LoD for the TSI Reporter has been determined to be 89.14 %SRR based on the calculations presented in CLSI EP17A.

2. **Interference by Endogenous Substances**
   Interference was not observed in TSI-containing human serum that was spiked with
   - bilirubin up to 36.6 mg/dL,
   - hemoglobin up to 250 mg/dL and
   - lipids up to 1,168 mg/dL.

3. **Cross-reactivity by Glycoprotein Hormones**
   - luteinizing hormone up to 625 mIU/mL,
   - human chorionic gonadatrophin up to 40,625 mIU/mL,
   - follicle stimulating hormone up to 2,000 mIU/mL,
   - thyroid stimulating hormone up to 0.35 mIU/mL.

4. **Cross-reactivity with Other Autoantibodies**
   The TSI Reporter was tested on 36 samples with autoimmune diseases other than GD:
   - 16 autoimmune hypothyroidism (Hashimotos' disease [Hm]),
   - 10 Rheumatoid Arthritis (RA) and
   - 10 Systemic Lupus Erythematosus (SLE).

   One (1) Hm sample tested positive for TSI, however, this is a sample with TSH levels at or near the level of interference reported above. All other samples tested negative.

5. Assay Cutoff

Patient serum, when tested using the TSI Reporter, will be considered positive for the presence of TSI if the resultant SRR% measures greater than or equal to (≥) 140% over the Reference Control. This is a preliminary cut-off which was established using a "training-set" of samples and was confirmed in the clinical studies. The TSI Reporter prototype device was tested for assay cutoff limits through testing of 30 subjects with diagnosed Graves' disease and 44 normal subjects with no known or clinically diagnosed thyroid disease. The SRR% data obtained for each of these subjects were analyzed using receiver operating characteristic (ROC) curve analysis.

Using a "testing-set" of samples, the preliminary cutoff was verified in pre-clinical testing with an additional 50 GD positive sera obtained from physicians with diagnostic information and 140 normal sera. Additional verification of the cutoff limit was performed through precision testing. A manufactured sample near the cutoff limit (≈ 157% above the Reference Control) was tested over 20 days (n=120) and yielded positive results ≥75% of the time and negative results ≤25% of the time. CLSI recommendations for assay cutoff verification (Evaluation Protocol (EP) 12-A, Section 7.0) are a 50%/50% positive to negative ratio for samples at the cutoff.

6. Intra-Assay Precision

The TSI Reporter was conducted by a single user over a 20 day period following the Precision Study Protocol (see Protocols and Procedures). Each sample was tested in triplicate wells and the resulting RLU values were averaged per sample. Each plate of cells analyzed per day contained 16 samples. The average variation for each sample was calculated (CV %) across each plate. It was calculated that the average intra-plate (n=16) variation (CV %) was 4.7%.

7. Inter-Assay Precision (Intra-Day)

The same samples tested during the Precision Study were analyzed for inter-assay precision within each day. Serum samples were manufactured to simulate a patient with high levels, medium levels and low levels of TSI by diluting serum from a known GD subject with highly stimulatory TSI into normal human serum and tested repeatedly. Serum from a TSI negative subject, TSI Positive Control and Reference Control were also tested. The total replicates for the types of samples tested per day were 6, 6, 6, 6, 2, and 4 for the high positive, medium positive, low positive, normal, TSI Positive Control and Reference Control respectively. The average triplicate was calculated per plate then analyzed for inter-plate variation. For the high TSI, medium TSI and low TSI containing serum, the average inter-assay CV% values calculated on day one of precision testing were 3.6%, 2.6% and 4.2% respectively. The Reference Control, TSI Positive Control and Normal Control were 2.4%, 5.0% and 5.0% respectively, with an overall inter-assay variation within this day calculated to be 3.8%.

8. Inter-Assay Precision (Inter-Day)

The data collected from the above studies were analyzed for inter-assay precision across a 20 day study. When combined, the average variation of the assay for the high TSI

containing serum, medium TSI containing serum, low TSI containing serum, normal serum, TSI Positive Control and Reference Control (n=120, n=120, n=120, n=120, n=40 and n=80) was 12%, 13%, 15%, 16%, 12% and 7% respectively, with an overall average inter-assay variation across 20 days calculated to be 12%.

9. Assay Reproducibility

To demonstrate competency with and reproducibility of the TSI Reporter, each trained site performed testing on the panel[a] described in Table 5.2 below twice a day over an eight day span:

Table 5.2: Reproducibility Panel Variation and Accuracy

Reagent	Volume	Expected Variation	Expected Accuracy
Specimen A	15x500 µL	+/- 15%	100%
Specimen B	15x500 µL	+/- 15%	100%
Specimen C	15x500 µL	+/- 25%	100%
Specimen D	15x500 µL	+/- 15%	50%

Table 5.3 reports the results for each of the sites over their proficiency training period. Site 3 requested 2 technicians be trained

Table 5.3: Proficiency Training Period Site Results (reported as Average SRR% and Coefficient of Variation)

Site 1-COH	Site 2 - MN	Site 3 - NC Technician 1	Site 3 - NC Technician 2
Sample A 270.09% 11.47%	Sample A 439.89% 10.51%	Sample A 280.10% 13.56%	Sample A 289.49% 10.55%
Sample B 293.11% 14.72%	Sample B 495.14% 14.59%	Sample B 374.26% 14.70%	Sample B 345.13% 11.80%
Sample C 48.23% 14.94%	Sample C 67.89% 14.66%	Sample C 44.33% 20.21%	Sample C 48.60% 22.46%
Sample D 158.6% 10.62%	Sample D 198.0% 10.96%	Sample D 143.6% 14.43%	Sample D 142.7% 12.12%

All sites performed the study using manufactured panel samples. Each site's data was analyzed cumulatively to determine the Reproducibility and Repeatability of the panel samples. Samples A and B both had a positive ratio (Number Positive/Total Number

[a] The panel consisted of four specimens created to meet the requirements for precision as set forth in both CLSI (EP12-A2) and FDA guidance documents (http://www.fda.gov/cdrh/ode/odecl051.html)

Tested) of 180/180, Sample C had a negative ratio (Number Negative/Total Number Tested) of 180/180 and Sample D had a positive ratio (Number Positive/Total Number Tested) of 139/180. The overall coefficient of variation (CV) % for Samples A, B, C and D were 23.7%, 23.7%, 24.6% and 17.9% respectively.

An additional smaller study was performed using three samples near the cut off was performed at two sites twice a day for five-days.

Table 5.4: Reproducibility Panel 2 Variation and Accuracy

Reagent	Volume	Expected Variation	Expected Accuracy
Specimen E	15x500 µL	+/- 15%	100%
Specimen F	15x500 µL	+/- 25%	100%
Specimen G	15x500 µL	+/- 25%	100%

Table 5.5 reports the results for each of the two sites over their proficiency training period.

Table 5.5: Reproducibility Results (reported as Average SRR% and Coefficient of Variation)

2-MN		Diagnostic Hybrids Inc.	
Specimen E		Specimen E	
194%	9.3%	170%	17.7%
Specimen F		Specimen F	
106%	20.2%	106%	20.7%
Specimen G		Specimen G	
102%	21.6%	107%	19.4%

Each site's data was analyzed cumulatively to determine the Reproducibility and Repeatability of the panel samples. Sample E had a positive ratio (Number Positive/Total Number Tested) of 60/60, Samples F and G had negative ratios (Number Negative/Total Number Tested) of 60/60. The overall coefficient of variation (CV) % for Samples E, F and G were 15.0%, 20.3%, and 20.5% respectively.

10. Assay Stability

Stability was determined through repeated testing of the assay over three lots of materials. All assay studies are carried out according to the final written procedure found in the Instructions for Use. Assay results were evaluated as a ratio of the sample over the reference control and are compared to each previous data set throughout the

study. The data has been trended over time. The variation of the results [including standard deviations and coefficient of variation (%)] were reported over time along with the sample result. The TSI Reporter materials, reagents and components are stable and pass all stipulated acceptance criteria for a minimum of 12 months.

CLINICAL PERFORMANCE

Studies began at two testing sites with a total of 312 specimens to be evaluated by both the subject and comparator devices. One specimen was excluded due to insufficient quantity for testing. Twelve of these specimens were excluded from statistical analysis due to indeterminate results on the comparator device. The remaining 299 specimens were analyzed for positive and negative percent agreement.

The following table (Table 5.6) details summary results from study site 1-COH and 2-MN combined information.

Table 5.6: Sites 1-COH and 2-MN Combined Result Summary

299 specimen results		Comparator Device (KRONUS TRAb)		
		+	-	Indeterminate
Subject Device (TSI Reporter)	+	**120**	**18**	**7**
	-	**8**	**153**	**5**
		95% Confidence Interval		
Positive Percent Agreement	93.8%	88.2 to 96.8%		
Negative Percent Agreement	89.5%	84.0 to 93.2%		

The ability of the subject device to detect TSI using a cell-based system was compared to the comparator device's radioreceptor assay. The positive percent agreement for study sites 1-COH and 2-MN combined was 93.8% (95% CI range 88.2% to 96.8%). The negative percent agreement for study sites 1-COH and 2-MN combined was 89.5% (95% CI range 84.0% to 93.2%). The performance data for the two devices are comparable and, therefore it appears that the TSI Reporter is substantially equivalent to the KRONUS TRAb.

An additional study was performed at a third testing site using 247 specimens. Sixteen of these specimens were excluded from statistical analysis due to indeterminate results on the comparator device. The remaining 231 specimens were analyzed for positive and negative percent agreement.

The following table (Table 5.7) details summary results from study site 3-NC.

Table 5.7: Site 3-NC Result Summary

231 specimen results		Comparator Device (KRONUS TRAb)		
		+	-	Indeterminate
Subject Device (TSI Reporter)	+	**53**	**4**	**2**
	-	**18**	**156**	**14**
		95% Confidence Interval		

Positive Percent Agreement	74.6%	63.5 to 83.3%
Negative Percent Agreement	97.5%	93.8 to 99.0%

The positive percent agreement was 74.6% (95% CI range 63.5% to 83.3%). The negative percent agreement was 97.5% (95% CI range 93.8% to 99.0%). An explanation for this decreased positive percent agreement value is the specificity difference between TSI Reporter and the comparator device. The comparator device detects autoantibodies to the TSHR, of which there are two classes, stimulating (TSI-hyperthyroidism) and blocking (TBI-hypothyroidism). The comparator device is unable to distinguish between the two antibody types, as stated in the Assay Limitations of the comparator device's product insert. TSI Reporter detects only TSI. Thus, during our clinical performance testing, it is likely that some patient sera have TBI and would be positive by the comparator device but negative by the subject device. There currently is no TBI specific cleared device and, as such, it is not possible to further analyze the discordant results. There is no published data indicating the prevalence of TBI in the normal population.

The patient TSH results from the 18 discordant specimens (Comparator Device positive/Subject Device negative) at study site 3-NC were further reviewed. The results indicate that 6 patients meet the ATA definition of hypothyroidism (>3.0). If these patients are removed from the dataset from site 3 the PPA increases to 81.5% (95% CI range 70.4% to 89.1%).

Study Site 3-NC is a reference laboratory that receives specimens from all medical disciplines to be tested as part of a thyroid screening panel. The testing of patients with hypothyroidism is more likely in a screening environment than at Sites 1-COH and 2-MN that service targeted disciplines (i.e. Endocrinologists and Thyroidologists). ATA guidelines defines Hypothyroidism as a high TSH level (>3.0). A review of the TSH levels from the three sites indicate a greater percentage of Hypothyroid patients were in fact evaluated at Study Site 3-NC (24% of specimens) than at Study Sites 1-COH and 2-MN (15% and 11%, respectively).

There was good correlation between the three study sites regarding negative percent agreement.

CLINICAL SENSITIVITY AND SPECIFICITY

The Clinical Sensitivity and Specificity for the device was determined by testing 249 characterized specimens. The data is presented in the table 5.8 below.

Table 5.8: Clinical Sensitivity and Specificity

		Diagnosis		
		Positives (Graves Disease)	Negative (Other autoimmune diseases and healthy controls)	Totals
TSI Reporter	Positive	46	1	47
	Negative	4	198	202
	Total	50	199	

Clinical Sensitivity: 92.0% (46/50)
Clinical Specificity: 99.5% (198/199)

CONCLUSION

Evidence presented in the above discussion supports substantial equivalence of the subject device, The Thyretain ™ TSI Reporter BioAssay (TSI Reporter), to KRONUS TSH Receptor Antibody (TRAb) Coated Tube (CT) Assay Kit, a device regulated under 21 CFR § 866.5870, product code JZO.

[1] Tahara K., Ban T., Minegishi T., Kohn L.D. Immunoglobulins from Graves' disease patients interact with different sites on TSH receptor/LH-CG receptor chimeras than either TSH or immunoglobulins from idiopathic myxedema patients. *Biochem Biophys Res Commun.* 1991 Aug 30; 179(1):70-7

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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MAY 21 2009

DIAGNOSTIC HYBRIDS Inc
c/o Ms. Gail R. Goodrum
Vice President of Regulatory Affairs
1055 East State Street
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Re: k083391
Trade/Device Name: Thyretain™ TSI Reporter BioAssay
Regulation Number: 21 CFR §866.5870
Regulation Name: Thyroid autoantibody immunological test system
Regulatory Class: Class II
Product Code: JZO
Dated: May 7, 2009
Received: May 08, 2009

Dear Ms. Goodrum:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter

will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (240) 276-0450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding postmarket surveillance, please contact CDRH's Office of Surveillance and Biometric's (OSB's) Division of Postmarket Surveillance at (240) 276-3474. For questions regarding the reporting of device adverse events (Medical Device Reporting (MDR)), please contact the Division of Surveillance Systems at (240) 276-3464. For more information regarding the reporting of adverse events, please go to http://www.fda.gov/cdrh/mdr/.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (240) 276-3150 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Maria M Chan

Maria M. Chan, Ph.D.
Director
Division of Immunology and Hematology Devices
Office of In Vitro Diagnostic Device Evaluation and Safety
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Enclosure

Indication for Use

510(k) Number (if known): k083391

Device Name: Thyretain ™ TSI Reporter BioAssay

Indication For Use:

The Thyretain ™ TSI Reporter BioAssay is intended for the qualitative detection in serum of thyroid stimulating autoantibodies to the thyroid stimulating hormone (TSH) receptors (TSHRs) on the thyroid. The detection of these stimulating autoantibodies, in conjunction with other clinical and laboratory findings, may be useful as an aid in the differential diagnosis of patients with Graves' disease (GD).

Prescription Use ✓ (21 CFR Part 801 Subpart D) And/Or Over the Counter Use ____. (21 CFR Part 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE; CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD)

Maria M. Chan

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) K083391